Rapid Detection of Norwalk-Like Viruses (NLVs)

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Contents

Abstract

NLVs (Norwalk-like viruses) are members of the caliciviridae family and are common causes of epidemic gastroenteritis characterized by short (24-48 hours) incubation and duration. They are currently under diagnosed because of limited availability of convenient diagnostic tests e.g. cell culture systems are not available for this group of viruses. Recent advances including the cloning and sequencing of several strains of this virus has enabled the detection of NLVs in faecal samples. This study describes the development of an RT-PCR and an enzyme immunoassay for the detection of an Australian NLV group 2b virus (Camberwell) and related viruses and their application to clinical samples.

A recombinant baculovirus containing the complete Camberwell capsid protein was provided by Assoc. Prof. Peter Wright (Monash University). Virus-like particles (VLPs) produced by this construct were purified and used to produce hyperimmune rabbit and guinea pig antisera for use in an antigen capture enzyme immunoassay (EIA) with a sensitivity of 0.1ng of purified VLPs. No cross-reaction was observed when a range of gastroenteritis causing bacteria and viruses were used to test the CAM EIA. When applied to a clinical outbreak 31/65 (48%) specimens were found to be EIA positive. In contrast 1000 consecutive routine specimens submitted to our laboratory were EIA negative.

A one tube RT-PCR was also developed for NLV Group 2 (Camberwell-like) viruses. Due to the non-availability of known NLV positive specimens in preliminary experiments, stock cultures of poliovirus and rotavirus positive (by EIA) faecal samples were used for initial optimization of the RT-PCR. The sensitivity of RT-PCR for the three viruses was determined to be 16 pfu for

poliovirus, *ca* 2.5 x 10^2 viral particles/ml for rotavirus and 2 x 10^4 copies of *in vitro* transcribed NLV RNA. However, when the outbreak faeces were tested only 24 of the 65 (37%) outbreak specimens were positive on first round NLV RT-PCR.

Further optimization including an improved faecal RNA extraction method (Qiagen RNeasy Plant RNA kit) and concentration of NLVs by Immuno and PEG precipitation methods all improved the sensitivity of the first round RT-PCR but failed to find any additional positive outbreak specimens. However, a nested PCR was found to increase the sensitivity of NLV RT-PCR by 100 fold *in vitro* and found an additional 6 EIA positive specimens to be nested PCR positive. None of the EIA negative specimens became positive. Sequencing of all RT-PCR and nested PCR products showed that they were all identical and showed 96% homology to the Camberwell strain.

This study suggests that NLV antigen capture EIA and RT-PCR assays may be of similar sensitivity and can provide complementary information during the investigation of outbreaks. The development of EIAs for representative strains of other NLV (and Sapporo Like Viruses) genogroups may therefore permit the production of clinically useful diagnostic EIAs. Further development of the RT-PCR assays will require the use of consensus primers for each of the NLV groups 1, 2 and SLV viruses.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma and to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made in the text. In accordance with the University of Adelaide regulations, I give my consent to this thesis being made available for photocopying and loan if accepted for the award of the degree.

Satiya Wati

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Abbreviations

ca	approximately
e.g.	for example
i.e.	that is
>	greater than
°C	degrees Celsius
bp	base pair
CAM	Camberwell
cDNA	complementary DNA
dATP	2'-deoxyadenosine 5'-triphosphate
DDW	double distilled water
dCTP	2'-deoxycytidine 5'-triphosphate
dGTP	2'-deoxyguanosine 5'-triphosphate
DIG	digoxigenin
DNA	deoxyribonucleic acid
DNaseI	deoxyribonuclease-1
dNTP	2'-deoxynucleoside 5'-triphosphate
DTT	dithiothreitol
dTTP	2'-deoxythymidine 5'-triphosphate
EIA	enzyme immunoassay
EDTA	ethylenediaminetetraacetic acid
EM	electron microscopy
FBS	fetal bovine serum
g (units)	gravity force
IEM	Immune electron microscopy
kb	kilobase pair
kDA	kilodalton
LB	Luria-Bertani medium
µg	microgram
µl	microlitre
µM	micromole per litre (micromolar)
mg	milligram

min(s)	minute(s)
ml	millilitre
mM	millimole per litre (millimolar)
MOPS	3-[N-morpholino]propanesulfonic acid
MW	molecular weight
ng	nanogram
nm	nanometre
NLV	Norwalk-like virus
NV	Norwalk virus
OD	optical density
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
pBS	plasmid Bluescript (SK)
PCR	polymerase chain reaction
PEG	polyethylene glycol
p.i.	post infection
ppm	parts per million
ppt	precipitation
RIA	radioimmunoassay
RNA	ribonucleic acid
RNase A	ribonuclease A
rNTP	ribonucleoside 5'-triphosphate
rRNA	ribosomal RNA
rpm	revolutions per minute
RT	reverse transcriptase
SDS	sodium dodecyl sulfate
SLV	Sapporo-like virus
SSC	standard saline citrate
TCA	trichloroacetic acid
UTP	uridine 5'-triphosphate
VLPs	virus-like particles
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactoside